Exhibit A

1: Pancreas 1989;4(3):282-8

Related Articles, Books

Peptide YY: intrapancreatic localization and effects on insulin and glucagon secretion in the mouse.

Bottcher G, Ahren B, Lundquist I, Sundler F.

Department of Medical Cell Research, Lund University, Sweden.

We studied the intrapancreatic localization of peptide YY (PYY) and the effects of PYY on insulin and glucagon secretion in the mouse. Immunofluorescence staining of mouse pancreatic tissue showed that PYY occurred within islet cells. These cells were located preferentially at the periphery of the islets. Sequential and simultaneous double immunostaining revealed that most PYY cells also displayed glucagon immunoreactivity; some PYY cells contained immunoreactive pancreatic polypeptide (PP). At the electromicroscopic level, PYY immunoreactivity was demonstrated within the secretory granules of both glucagon cells and of a small granular cell type, which showed structural similarities to PP cells. In in vivo experiments, PYY at dose levels between 0.53 and 8.5 nmol/kg had no influence on basal plasma levels of insulin, glucagon, or glucose. In contrast, insulin secretion stimulated by glucose or the cholinergic agonist carbachol was inhibited by PYY (by 33 and 26%, respectively, at 4.25 nmol/kg). Similarly, carbachol-induced glucagon secretion was inhibited by PYY (by 47% at 4.25 nmol/kg). We conclude that PYY occurs in islet cells of the mouse pancreas, most of which are glucagon cells, and that PYY inhibits stimulated insulin and glucagon secretion in vivo in the mouse.

PMID: 2660131 [PubMed - indexed for MEDLINE]

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1: Am J Surg 1996 Jan;171(1):192-6

Adjuvant hormonal treatment with peptide YY or its analog decreases human pancreatic carcinoma growth.

Liu CD, Rongione AJ, Garvey L, Balasubramaniam A, McFadden DW.

Department of Surgery, UCLA Center for Health Sciences, USA.

BACKGROUND: Recent studies have revealed decreased pancreatic cancer cell growth upon administration of peptide YY (PYY). We examined whether adjuvant treatment with PYY or its synthetic analog, BIM-43004, would decrease human pancreatic adenocarcinoma growth. MATERIALS AND METHODS: Human pancreatic ductal adenocarcinomas, MiaPaCa-2 and BxPC-3, were cultured and assessed for growth by MTT assay. Pancreatic cancer cells received 500 pmol of PYY or BIM-43004 for 24 hours prior to 5-fluorouracil (5-FU; 10 micrograms/mL) and leucovorin (40 micrograms/mL) administration. Cell membrane epidermal growth factor (EGF) receptors were analyzed by Western blotting after exposure to peptides and chemotherapy. RESULTS: Cancer cell growth was reduced in all groups receiving hormonal pretreatment (23% PYY/5-FU/leucovorin versus control; 27% BIM-43004/5-FU/leucovorin versus control) as compared with groups receiving 5-FU and leucovorin only (16% versus control). The EGF receptor expression was reduced by 30% in cells treated with PYY/5-FU/leucovorin and by 45% in cells treated with BIM/5-FU/leucovorin as compared with control cells without treatment. CONCLUSION: Human pancreatic cancer cell growth is further decreased when pretreated with PYY or its synthetic analog prior to chemotherapy.

PMID: 8554139 [PubMed - indexed for MEDLINE]

1: Dig Dis Sci 1999 Mar;44(3):643-8

Characterization of two novel proabsorptive peptide YY analogs, BIM-43073D and BIM-43004C.

Litvak DA, Iseki H, Evers BM, Greeley GH Jr, Hellmich MR, Iwase K, Balasubramaniam A, Townsend CM Jr.

Department of Surgery, The University of Texas Medical Branch, Galveston 77555-0527, USA.

Effective clinical therapy to augment intestinal absorption of water and electrolytes does not exist; the gut hormone, peptide YY (PYY), is a potent proabsorptive agent in animal models. The purpose of our study was to evaluate the effects of two novel PYY analogs, BIM-43073D and BIM-43004C, on intestinal absorption. Dogs with ileal Thiry-Vella fistulae (TVF) were treated with either PYY, BIM-43073D, or BIM-43004C. Administration of BIM-43073D significantly increased water and sodium absorption over baseline and maintained this level of increased absorption for a longer duration than an equimolar dose of PYY. Administration of BIM-43004C significantly increased sodium and water absorption over baseline at a level equal to that of PYY. The novel PYY analogs, BIM-43073D and BIM-43004C, are effective proabsorptive agents with BIM-43073D producing more sustained effects than PYY. These compounds may be clinically useful in the treatment of gut malabsorption in conditions such as cholera, Crohn's disease, and the short-bowel syndrome.

PMID: 10080163 [PubMed - indexed for MEDLINE]

1: Dis Colon Rectum 1997 Apr;40(4):478-82 Related Articles, Books

Intraluminal peptide YY induces colonic absorption in vivo.

Liu CD, Newton TR, Zinner MJ, Ashley SW, McFadden DW.

Department of Surgery, UCLA Center for the Health Sciences and Sepulveda Veterans Administration Medical Center, Los Angeles, California, USA.

INTRODUCTION: Peptide YY (PYY) is a 36 amino acid hormone released into the circulation and lumen of the intestine after a meal. Previous studies have shown that exogenous administration of intravenous PYY stimulates water and electrolyte absorption in both the small and large intestines. The purpose of this study was to examine the effects of intraluminal administration of PYY on colonic absorption of electrolytes and water. METHODS: Six conditioned 25-kg dogs had 20 cm of colonic Thiry-Vella fistulae surgically constructed under general anesthesia. After a two-week recovery period, the animals received intraluminal PYY at 600 pmol/kg/hour after a 90-minute steady-state basal period. The Thiry-Vella fistulae were perfused with an isotonic buffer solution containing [14C]polyethylene glycol as a volume marker. Ion and water transport were measured every 15 minutes. RESULTS: On intraluminal infusion of PYY, increased absorption of water, sodium, and chloride was observed in the colon. A twofold increase in absorption rates occurred compared with basal rates lasting more than one hour after cessation of intraluminal PYY ($N = 6$; $P < 0.05$ vs. basal by analysis of variance). CONCLUSION: PYY-secreting cells of the colon may contribute to the regulation of absorption after a meal. Exogenous administration of intraluminal PYY may also be a therapeutic treatment modality for malabsorption.

PMID: 9106700 [PubMed - indexed for MEDLINE]

1: Gastroenterology 1993 Nov;105(5):1441-8 Related Articles, Books

Peptide YY is a physiological regulator of water and electrolyte absorption in the canine small bowel in vivo.

Bilchik AJ, Hines OJ, Adrian TE, McFadden DW, Berger JJ, Zinner MJ, Ashley SW.

Department of Surgery, UCLA School of Medicine.

BACKGROUND: Peptide YY (PYY), a hormone released following a meal, is one potential mediator of intestinal absorption. Although PYY inhibits 5'-cyclic adenosine monophosphate (cAMP)-stimulated small intestinal secretion in vitro, its effects on fluid and electrolyte transport in vivo are unknown. METHODS: This study examines the effects of physiological doses of PYY in dogs (n = 6) with jejunal and ileal exteriorized, neurovascularly intact intestinal loops (Thiry-Vella fistulas). RESULTS: Plasma PYY levels increased after a meal from 155 +/- 15 to 324 +/- 26 pmol/L at 30 minutes and remained elevated for 2 hours. PYY infused intravenously in unfed animals at 25, 50, 100, and 200 pmol.kg-1.h-1, produced a dose-dependent increase in plasma PYY levels. At 100 pmol.kg-1.h-1, PYY plasma concentrations were similar to those of fed animals (317 +/- 39 pmol/L). PYY infusion resulted in a dose-dependent increase in water and electrolyte absorption at all doses in both the jejunum and ileum. Although the relative increase in absorption was similar, the magnitude was greater in the ileum. CONCLUSIONS: Physiological concentrations of PYY produced an increase in small bowel absorption of water and electrolytes in vivo. The postprandial release of PYY may mediate the increase in absorption following a meal. Such a proabsorptive agent may have considerable potential for clinical use in malabsorptive states.

PMID: 8224646 [PubMed - indexed for MEDLINE]

1: Pancreas 1995 Mar;10(2):123-30 Related Articles, Books

Differential effects of peptide YY, neuropeptide Y, and sigma ligands on neurally stimulated external pancreatic secretion in the rat.

Nagain C, Chariot J, Roze C.

INSERM U410, Faculte de Medecine X. Bichat, Paris, France.

The endocrine peptide YY (PYY) inhibits pancreatic secretion in animals and in man through indirect pathways. Neuropeptide Y (NPY), whose chemical structure is very close, displays similar effects. Recently, sigma ligands were shown to produce in vivo several neural pharmacologic effects that seemed indistinguishable from those of NPY. This might occur by interaction with the same (or closely related) receptors or by activation of a common final pathway. The purpose of the present work was to test whether PYY, NPY, and sigma agonists also display closely related activities on pancreatic secretion. The sigma ligands (+)-N-allyl normetazocine (d-NANM) and di(ortho-tolyl) guanidine (DTG) were used. Pancreatic secretion was stimulated by the centrally acting agent 2-deoxyglucose (2DG) in anesthetized rats. The rats were also administered either an infusion of peptide (PYY: 25-250 pmol/kg/h, NPY: 75-750 pmol/kg/h), continued for 2 h, or a bolus injection of d-NANM (3 mg/kg) or DTG (1 mg/kg). In antagonist experiments, the dopamine and sigma antagonist haloperidol (1 mg/kg, i.v.), the adrenoceptor antagonists idazoxan (0.3 mg/kg, s.c.), prazosin (0.5 mg/kg, s.c.), propranolol (1 mg/kg, s.c.) and the opiate receptor antagonist naloxone (1 mg/kg, s.c.) were injected, 5 min before the peptide infusion had begun. Neither PYY nor NPY changed basal pancreatic secretion. PYY and NPY produced a dose-related inhibition of 2DG-stimulated pancreatic secretion. The observed inhibition after 250 pmol/kg/h of PYY was volume, 78% ($p < 0.01$); bicarbonate, 84% ($p < 0.01$); protein, 78% ($p < 0.01$); whereas the physiologically relevant dose of 25 pmol/kg/h induced approximately 30% inhibition of these variables. (ABSTRACT TRUNCATED AT 250 WORDS)

PMID: 7716135 [PubMed - indexed for MEDLINE]

Peptide YY inhibits vasopressin-stimulated chloride secretion in inner medullary collecting duct cells

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Department of Cell Biology, Duke University Medical Center and Division of Gastroenterology, Department of Veterans Affairs Medical Center, Durham, North Carolina 27710; and Department of Pharmacology and Physiology, Oklahoma State University, College of Osteopathic Medicine, Tulsa, Oklahoma 74107

Breen, Christopher M., Peter J. Mannon, and Bruce A. Benjamin. Peptide YY inhibits vasopressin-stimulated chloride secretion in inner medullary collecting duct cells. *Am. J. Physiol.* 275 (*Renal Physiol.* 44): F452–F457. 1998. mIMCD-k2 cells are derived from the inner medullary collecting duct of a mouse and exhibit electrogenic sodium absorption and cAMP- and vasopressin (AVP)-stimulated electrogenic chloride secretion [N. L. Kizer, B. Lewis, and B. A. Stanton. *Am. J. Physiol.* 268 (*Renal Fluid Electrolyte Physiol.* 37): F347–F355. 1995; and N. L. Kizer, D. Vandorpe, B. Lewis, B. Bunting, J. Russell, and B. A. Stanton. *Am. J. Physiol.* 268 (*Renal Fluid Electrolyte Physiol.* 37): F854–F861, 1995]. The purpose of the present study was to determine how peptide YY (PYY) affects electrogenic Na^+ and Cl^- current in mIMCD-k2 cells. Short-circuit currents (I_{sc}) were measured across monolayers of mIMCD-k2 cells mounted in Ussing-type chambers. PYY did not alter baseline I_{sc}, nor did it alter I_{sc} in chloride-free conditions, indicating no effect on electrogenic sodium transport. Baseline chloride current in these cells is low; therefore, chloride short-circuit current (I_{sc}^{Cl}) was stimulated with AVP (10 nM) added to the basolateral surface and 10 μM amiloride added to the apical surface. Although apical applications of PYY had no effect, basolateral application of PYY caused attenuation of I_{sc}^{Cl}, with the maximal inhibitory dose (100 nM) causing 52 ± 1.3% inhibition (IC_{50} = 0.11 nM). Inhibition by PYY of I_{sc}^{Cl} is mediated through the Y_2 receptor subtype, as PYY-(3–36) was the only PYY analog tested that caused inhibition and was equipotent to PYY. Inhibition by PYY of I_{sc}^{Cl} was abolished following incubation with pertussis toxin. We also show that PYY inhibits AVP-stimulated cAMP accumulation, with a maximal inhibitory dose (100 nM) causing a 38% ± 6% inhibition (IC_{50} = 0.16 nM), comparable to inhibition by PYY of I_{sc}^{Cl}. We conclude that PYY acts through either G_i or G_o to inhibit adenylate cyclase activity, leading to a decrease in AVP-stimulated chloride current.

short-circuit current; chloride secretion; arginine vasopressin; adenosine 3′,5′-cyclic monophosphate

PEPTIDE YY (PYY) is a 36-amino acid peptide first isolated from the porcine intestine (36). It has subsequently been identified in the intestinal mucosa of ileum, colon, and rectum in many species including human, dog, rat, and rabbit (13, 14, 22, 23, 25, 35–37). PYY has strong homology with both neuropeptide Y (NPY) and pancreatic polypeptide, with which it shares certain common receptors.

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PYY is released into the circulation by the gut, primarily in response to feeding (30, 38). PYY-(3–36), which is a specific agonist for the Y_2 receptor, appears to form a significant amount of PYY circulating postprandially (17). Although most studies concerning the action of PYY have focused on the gastrointestinal system, both Y_1 and Y_2 receptors for PYY have been demonstrated in the kidney (8, 26, 34). Ohtomo et al. (28) have shown that the Y_2-specific agonist NPY-(13–36) stimulates Na^+-K^+-ATPase activity in the renal proximal tubule. PYY and NPY also affect renal vascular function, with these effects being associated with the Y_1 receptor subtype (6, 26; C. A. Blaze, S. R. Vigna, P. J. Mannon, A. R. Kherani, and B. A. Benjamin, unpublished observations). These findings suggest that the gut may play a role in modulating renal function in the postprandial state.

Preliminary studies in our lab demonstrated the presence of NPY/PYY receptors on renal epithelial mIMCD-k2 cells. These cells are derived from the initial segment of the mouse inner medullary collecting duct (IMCD) and exhibit cAMP- and vasopressin (AVP)-stimulated chloride secretion (20, 21). PYY receptors are coupled to cyclase inhibition and have been shown to inhibit cAMP-dependent chloride secretion (3, 10). The purpose of the present study was to determine the effect of PYY on basal and AVP-stimulated chloride secretion in mIMCD-k2 cells. Results from these studies demonstrate that PYY inhibits AVP- and cAMP-stimulated chloride secretion in the renal epithelial cell model, mIMCD-k2 cells.

METHODS

mIMCD-k2 cells were provided by Dr. B. A. Stanton.

Cell culture. mIMCD-k2 cells were cultured in tissue culture flasks coated with Vitrogen plating medium containing human fibronectin (1 mg/ml), 1% Vitrogen 100, and placed in an incubator maintained at 37°C and gassed with 5% CO_2-95% air. The cells were grown in DMEM supplemented with 1 nM aldosterone, 5% fetal bovine serum, 2 mM L-glutamine, 50 U/ml penicillin, and 50 μg/ml streptomycin. Medium was changed every 48 h.

Permeable supports. mIMCD-k2 cells were harvested from confluent culture flasks by trypsinization (0.05% in HBSS) and reseeded onto 24-mm polycarbonate membranes (Costar). The medium was changed every 48 h. Electrical resistance of cell monolayers was monitored using chopstick electrodes (EVOM). All experiments were performed on membranes from the same seeding.

Measurement of short-circuit current. Short-circuit experiments were performed using membranes whose resistance was 400–800 Ω (4.7 cm^2 growth area, 1,500 Ω·cm^2), as

measured by chopstick electrodes. Short-circuit current (I_{sc}) was measured by placing Transwell membranes in an World Precision Instruments (WPI) Ussing-type chamber. Voltage was clamped to 0 mV with a WPI DVC 1000 voltage clamp. Bath solutions were maintained at 37°C. Solutions were circulated by gas lift using 5% CO_2-95% air. Electrical connections from bath to voltage clamp were made with 3 M KCl-5% agar bridges and Ag-AgCl wires. Positive current represents the net flow of cations from the apical to basolateral bath solutions or the net flow of anions from the basolateral to apical bath solutions. Current output was digitized by a MacLab analog-digital converter and stored on a Macintosh SE computer.

Solutions. Most experiments were done using DMEM as the perfusate. For chloride-free experiments, the following solution was used: 24 mM $NaHCO_3$, 114 mM sodium isethionate, 3 mM $KHCO_3$, 2 mM $MgSO_4$, 0.5 mM $CaSO_4$, 8 mM HEPES, and 5 mM glucose, and adjusted to pH 7.4. In studies to determine chloride current, 50 μM amiloride was added to the apical bath to inhibit sodium channels.

Pertussis toxin sensitivity. mIMCD-k2 monolayers grown on permeable supports were incubated overnight in DMEM culture medium containing 100 ng/ml pertussis toxin (PTX). Membranes were then placed in the Ussing chamber, and chloride secretion was stimulated by the addition of 10 nM AVP under short-circuit conditions.

cAMP assay. Cellular cAMP levels were determined using a nonradioactive cAMP assay kit (Amersham). Confluent monolayers grown on permeable supports were preincubated for 30 min in 0.5 mM IBMX in DMEM prior to the addition of PYY (10^{-8}–10^{-11} M). Following 5-min incubation with PYY, AVP (10 nM) was added. Following a 15-min incubation in the presence of AVP, the medium was removed, cAMP was extracted in two volumes of ice-cold 65% ethanol, and the samples were then dried down in a vacuum oven prior to resuspension in assay buffer.

Statistics and analysis. Differences between means were compared with Student's *t*-test or analysis of variance followed by Dunnett's multiple comparisons test (dose-response data; Instat). Curve fits were done using Graphpad Prism. All data are means ± SE.

Reagents. All peptides were purchased from Peninsula Laboratories. Amiloride, forskolin, and aldosterone were all purchased from Sigma Chemical. Sodium isethionate was purchased from Fluka Chemika. The sodium salt of 8-(4-chlorophenylthio)-cAMP (8-CPT-cAMP) was purchased from Calbiochem Biochemicals. 5-Nitro-2 (3 phenylpropylamino) benzoic acid (NPPB) was purchased from Research Biochemical International.

RESULTS

PYY effect on I_{sc}. PYY did not alter basal I_{sc} or I_{sc} in chloride-free conditions (data not shown), indicating no effect on active sodium transport. To stimulate chloride secretion, 10 nM AVP was added to the basolateral membrane, and 1 μM amiloride was added to the apical surface. Under these conditions, I_{sc} represents electrogenic chloride secretion (20, 21). Figure 1*A* shows the stimulated chloride current; the response to AVP is biphasic, with an initial peak followed by a prolonged plateau phase (>30 min). NPPB, a chloride channel blocker added to the apical membrane, inhibits I_{sc}, indicating that the current was due to the activity of chloride channels. Figure 1*B* shows the effect of PYY on AVP-stimulated chloride secretion. After the plateau

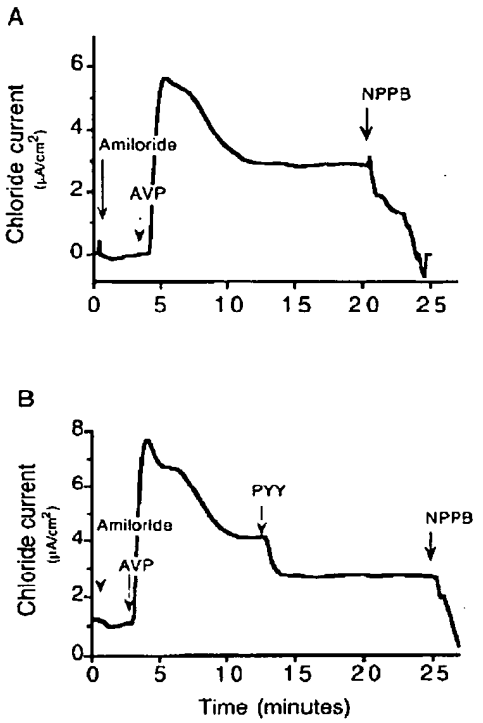

Fig. 1. *A*: representative short-circuit current (I_{sc}) showing the effect of 10 nM arginine vasopressin (AVP) added to the basolateral bath. Amiloride (10 μM) was added to the apical bath to inhibit electrogenic Na^+ reabsorption. 5-Nitro-2-(3-phenylpropylamino)benzoic acid (NPPB, 100 μM), a chloride channel blocker, was added to the apical bath to inhibit chloride secretion. *B*: representative experiment showing PYY attenuation of AVP-stimulated chloride secretion. PYY (10 nM) was added to the basolateral compartment.

phase was reached, addition of PYY to the basolateral membrane caused an attenuation of I_{sc}. Figure 2 shows the dose response for PYY inhibition of chloride current. This inhibition was expressed as percent inhibition of AVP-stimulated current, with 100% stimulation being the AVP plateau level minus the pre-AVP current. Maximal inhibitory doses of PYY (100 nM) caused a

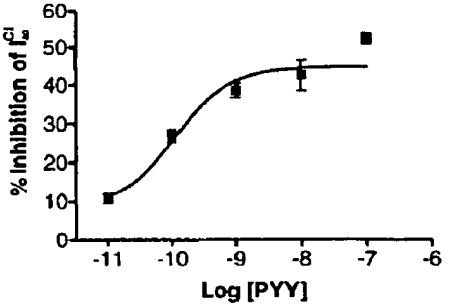

Fig. 2. Inhibition of AVP-stimulated I_{sc}^{Cl} by PYY as a function of the concentration of PYY. Values are expressed as percent inhibition, with 100% being the difference between I_{sc} prior to AVP addition and the plateau I_{sc} value at the time of PYY addition. $EC_{50} = 1.1 \times 10^{-10}$ M. Additions of 0.1 mM PYY caused a significant decrease in chloride short-circuit current (I_{sc}^{Cl}) ($P < 0.05$). Points are the average of 3 experiments ± SE.

52 ± 1.3% (mean ± SE, n = 3) inhibition of AVP-stimulated chloride current (IC_{50} = 0.11 nM). Additions of PYY > 0.1 nM caused a significant ($P < 0.05$) decrease in chloride short-circuit current (I_{sc}^{Cl}). To test whether order of peptide addition was important, monolayers were treated with PYY (10 nM) for 5 min followed by addition of AVP. This did not significantly alter the magnitude of inhibition by PYY of AVP-stimulated Cl secretion; i.e., AVP stimulation of chloride current after exposure to PYY attained only 60% of the plateau value in the absence of PYY. Addition of PYY to the apical membrane did not alter I_{sc} (data not shown).

Agonist profile. PYY is known to interact through a number of receptor subtypes. The following receptor analogs were added to the basolateral membrane to determine the receptor subtype responsible for the inhibition by PYY of chloride secretion: pancreatic polypeptide, [Leu^{31},Pro^{34}]NPY, and PYY-(3–36). A 10 nM dose was chosen because this concentration is ~100-fold greater than the IC_{50} value for the observed inhibition of I_{sc} and cAMP accumulation. PYY-(3–36) is a Y_2-receptor agonist with an IC_{50} for the Y_1 and Y_2 receptor of 810 nM (18) and 0.06 nM, respectively. [Leu^{31},Pro^{34}]NPY is a Y_1 agonist with IC_{50} values for the Y_1 and Y_2 receptor of 0.8 and 140 nM, respectively (4, 16). Pancreatic polypeptide is a Y_4 agonist with an IC_{50} value that exceeds 100 nM and 1,000 nM for the Y_1 and Y_2 receptors, respectively (24). Given the IC_{50} values for the Y_1, Y_2, and Y_4 agonists, the 10 nM dose of peptide is appropriate for determining the receptor subtype when using these three ligands in combination. At the 10 nM dose, PYY-(3–36) was the only analog that caused a change in I_{sc}^{Cl}. PYY (3–36) was found to be equipotent to PYY in inhibiting I_{sc}^{Cl} at 0.1 nM [11.1 ± 2.1% PYY vs. 14.5 ± 3% PYY-(3–36), n = 7], 1 nM [39.0 ± 2.1% PYY vs. 44.0 ± 2.6%, PYY-(3–36), n = 4], and 100 nM [52.3 ± 1.3% PYY vs. 46.2 ± 5.8% PYY-(3–36), n = 3].

Role of PYY in cAMP-dependent chloride secretion. Previous studies have shown that the cell-permeable cAMP analog 8-CPT-cAMP stimulates chloride secretion in mIMCD-k2 cells. The effect of PYY on 8-CPT-cAMP-stimulated chloride secretion was tested by the addition of 100 μM 8-CPT-cAMP to the apical reservoir, which stimulated chloride secretion; PYY (10 nM) was then added to the basolateral membrane. PYY did not attenuate 8-CPT-cAMP-stimulated chloride secretion (5.97 ± 0.34 μA/cm^2 pre-PYY vs. 5.97 ± 0.32 μA/cm^2 post-PYY, n = 4). The effect of PYY on forskolin-stimulated chloride secretion was also tested. Chloride current was stimulated by the addition of 1 μM forskolin to the apical membrane followed by addition of PYY (10 nM) to the basolateral membrane. Inhibition by PYY of forskolin-stimulated chloride secretion (50.2 ± 3.2% inhibition I_{sc}^{Cl}) was similar to its degree of inhibition of AVP-stimulated chloride secretion (42.7 ± 4.1% inhibition I_{sc}^{Cl}).

To test that PYY modulates cellular cAMP levels that may be mechanistically associated with its inhibition of cAMP-stimulated chloride secretion, cell monolayers were exposed to PYY (10^{-7}–10^{-12} M) for 4 min in the presence of IBMX (0.5 mM) prior to the addition of AVP (10 nM). Figure 3 shows the dose response for PYY inhibition of AVP-stimulated cAMP accumulation expressed as percent maximal cAMP accumulation. Maximal cAMP accumulation was the difference between AVP-treated cells (33 ± 1.5 pmol/mg protein) and basal cAMP levels (2.1 ± 0.23 pmol/mg protein). Addition of 0.1 nM PYY caused a significant ($P < 0.01$) reduction in cAMP levels, with maximal PYY (100 nM) causing 38 ± 6% inhibition of cAMP levels with an IC_{50} value of 0.16 nM.

PTX sensitivity. AVP is postulated to stimulate I_{sc}^{Cl} by increasing intracellular cAMP through G_s stimulation of adenylate cyclase. In other cell systems, PYY is known to decrease cAMP levels by G_i-mediated inhibition of adenylate cyclase. G_i inhibition is known to be sensitive to PTX. We therefore tested the effect of PTX on the attenuation by PYY of chloride current. mIMCD-k2 cells were incubated overnight in medium containing 100 ng/ml PTX. PTX incubation did not alter the ability of AVP to stimulate chloride current [2.32 ± 0.30 μA/cm^2 for AVP + PTX (n = 8) vs. 2.38 ± 0.26 μA/cm^2 for AVP alone (n = 6)]. Figure 4 shows the effect of PTX pretreatment on the attenuation by PYY of AVP-stimulated I_{sc}^{Cl}. In the absence of PTX, PYY (100 nM) attenuated chloride current by 49.3 ± 1.6%. In the presence of PTX, PYY had no effect on chloride current (0.5 ± 0.12%, 100 nM PYY + PTX, $P < 0.05$).

DISCUSSION

Previous characterization of mIMCD-k2 cells has shown that AVP-stimulated chloride secretion is due to increased cellular cAMP levels leading to activation of the cystic fibrosis transmembrane conductance regulator (CFTR) chloride channel (20, 21, 39). Here, we show that 100 nM PYY maximally inhibits AVP-stimulated chloride secretion by mIMCD-k2 cells by up to 52% (IC_{50} = 0.1 nM). PYY does not affect basal I_{sc} or alter I_{sc} in chloride-free conditions, indicating no effect on active sodium transport. The cell-permeable analog of cAMP, 8-CPT-cAMP, has also been shown to stimulate chloride secretion in mIMCD-k2 cells. Here, we show that PYY does not alter 8-CPT-cAMP-stimulated chlo-

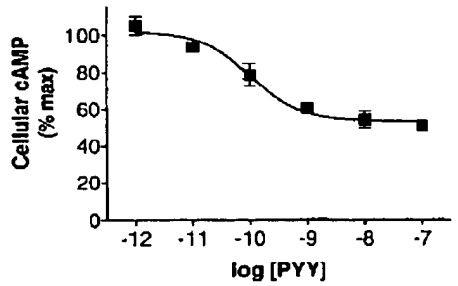

Fig. 3. Inhibition of AVP stimulated (10 nM) cAMP generation by PYY as a function of PYY concentration. Values are expressed as percent cellular maximum cAMP, with 100% being the difference between control (nonstimulated) and AVP-stimulated values. Points are the average of 3 experiments ± SE. All membranes were from the same seeding. Addition of 0.1 nM PYY caused a significant ($P < 0.05$) reduction in cAMP levels. IC_{50} = 0.15 nM.

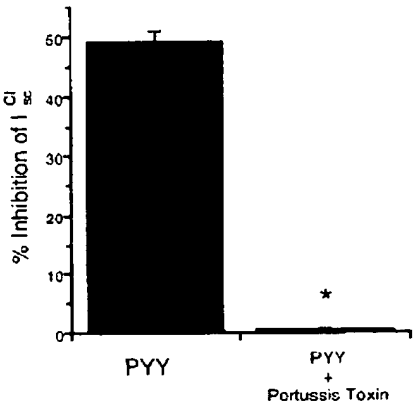

Fig. 4. Effect of preincubating mIMCD-k2 monolayers (~18 h) in medium containing pertussis toxin (100 ng/ml). The ability of PYY to attenuate AVP-stimulated chloride current was significantly reduced (49.3 ± 1.64%, 100 nM PYY, vs. 0.5 ± 0.12%, 100 nM PYY + pertussis toxin; * $P < 0.05$, $n = 5$). Pertussis toxin did not affect AVP-stimulated chloride secretion.

ride secretion but does inhibit forskolin-stimulated chloride secretion. These results are consistent with PYY acting at the level of adenylate cyclase to modulate cellular levels of cAMP.

When the effect of PYY on AVP-stimulated cAMP generation was tested, we found that PYY inhibits cAMP generation in a dose-dependent manner, with an IC_{50} of 0.16 nM, approximately equal to the 0.11 nM value for inhibition of chloride current. Maximal inhibitory doses of PYY (100 nM) caused a 38% inhibition of AVP-stimulated cAMP generation comparable with the inhibition by PYY of I_{sc}^{Cl}. The inhibition by PYY of I_{sc}^{Cl} was abolished by preincubation with PTX, consistent with PYY acting through G_i or G_o to inhibit adenylate cyclase. This is similar to the inhibition by PYY of chloride secretion in the gut (3, 10) and to the antagonism by NPY of AVP actions in rat cortical collecting tubules where NPY reduces AVP-stimulated hydraulic conductivity (11).

In the present study, we show that the attenuation by PYY of chloride secretion is mediated by the Y_2 receptor subtype that recognizes COOH-terminal fragments of PYY. The Y_2 receptor is the same subtype that is responsible for activation of the Na^+-K^+-ATPase activity in the renal proximal tubule of the rat (28, 29). Y_2 receptors (IC_{50} = 0.15 nM, PYY) have also been demonstrated in rabbit proximal tubule (34). We show that PYY attenuates chloride secretion with an IC_{50} of 0.1 nM. This value is within the range for plasma PYY levels, which rise postprandially to 0.4, 0.2, and 0.05 nM in dogs, rats, and humans respectively (1, 5, 15). In humans, the COOH-terminal fragment PYY-(3–36) accounts for 37% of PYY-like immunoreactivity in the fasting state and 63% in the postprandial state (17). Thus the postprandial increase in PYY levels contain significant amounts of peptide, which would specifically activate Y_2 receptors and potentially regulate renal tubular function specifically.

The physiological significance of chloride secretion by cells of the distal collecting duct, as modeled by mIMCD-k2 cells, is not well established. Electrogenic chloride secretion has been proposed as mechanism for NaCl secretion by the IMCD (20, 33). Chloride secretion is a mechanism for NaCl secretion by the shark rectal gland (19), which shares a number of features with mIMCD-k2 cells including cAMP-dependent chloride secretion via apical CFTRs, basolaterally located Na^+-K^+-ATPases, and basolaterally located Na-K-2Cl cotransporters (32, 39). Although the IMCD is normally associated with net reabsorption of NaCl, the ability of the IMCD to secrete sodium has been shown in microperfused IMCD tubules (33). Atrial natriuretic factor increased the electronegative lumen potential, and this increase in electronegativity was associated with increased bath-to-lumen chloride flux and NaCl secretion (33). AVP stimulates I_{sc}^{Cl} in mIMCD-k2 monolayers, indicating active Cl^- secretion; the effect on lumen negativity or Cl^- flux has not been studied, nor has the effect on net NaCl transport been studied. In colonic mucosa, inhibition of chloride secretion, by PYY under short-circuit conditions has been shown to increase net Na and Cl absorption without altering active sodium transport (27). It should be noted that in A6 cells, a *Xenopus* kidney cell line, vasotocin (the amphibian analog to AVP) has been shown to increase chloride current under short-circuit conditions (40). However, under open-circuit conditions, vasotocin treatment leads to a net uptake of NaCl. In A6 cells, AVP also causes a delayed increase in amiloride-sensitive current, and the increase in chloride secretion is thought to favor Na^+ uptake via the activated amiloride-sensitive Na^+ channels. In mIMCD-k2 cells, we do not see an increase in amiloride-sensitive current, even after 20 min of AVP treatment, nor do we see any increase in I_{sc} under chloride-free conditions. Thus, in mIMCD-k2 cells, AVP does not increase active transport of sodium necessary for the net increase in NaCl uptake. Although we cannot rule out chloride secretion leading to net NaCl reabsorption, the mechanism that mediates this in A6 cells does not appear to be active in mIMCD-k2 cells.

Because the collecting duct is the terminal segment of the nephron, increased chloride secretion in this segment would result in increased excretion of NaCl (20, 33); i.e., it would be natriuretic. We have shown that PYY inhibits chloride secretion and thus would tend to be antinatriuretic, consistent with its proposed action in the proximal tubule (2) also mediated through the Y_2 receptor subtype. This proposed mechanism whereby the inhibition by PYY of active chloride secretion leads to net NaCl absorption has been shown in the gut (27). However, the physiological significance of the actions of PYY on renal function is unclear. A number of in vivo PYY and NPY infusion studies have shown both antinatriuretic action in monkeys (12), as well as natriuretic effects in humans (31). Other studies have shown that PYY can be antinatriuretic when infused at doses that mimic physiological levels, but when infused at higher levels, PYY tended to be natriuretic (7). This report and other work (2, 28) suggest that at the

tubular level PYY might exert antinatriuretic effects. The disproportionate rise in Y_2 active PYY fragments seen postprandially may represent a mechanism to differentially regulate renal tubular activity without affecting renal vasculature.

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